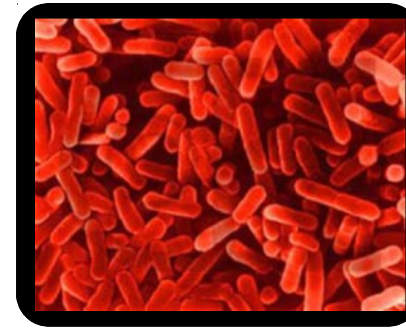
Intracellular nucleic acid sensing

Brian Ferguson

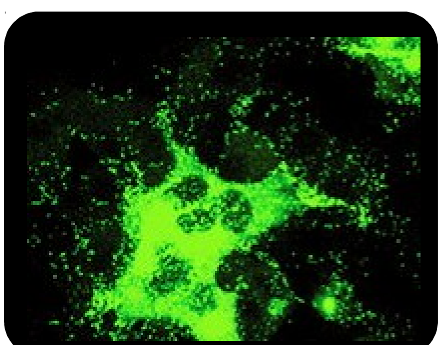
Part II Immunology

22/10/2103

Pattern recognition receptor signalling



1) Direct detection of pathogens:
   - Molecular discrimination between self and non-self - PAMPs
   - Sounds the infection 'alarm'
2) Detection of injury or tissue damage:
   - Inflammatory response to DAMPs
   - Restores homeostasis

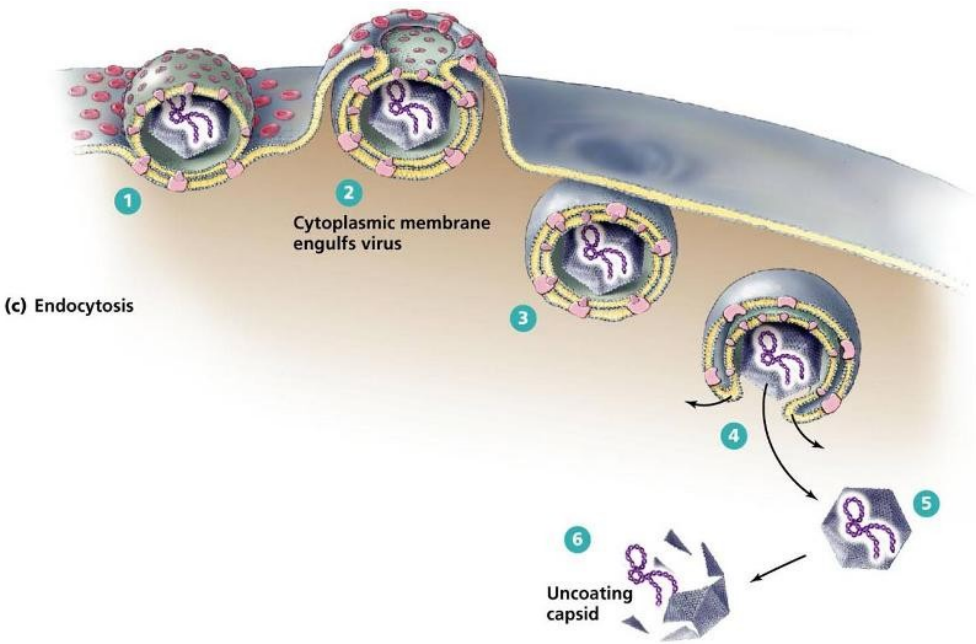


Require **specific sensors** but induce the **same mediators -** secretion of interferons, cytokines, chemokines , AMPs etc.

Intracellular nucleic acid sensing of infection

The production of type 1 inferferons in response to virus infection *in vivo* is critically dependent on **nucleic acid** sensing in non-heamatopoietic cells

Even though there are many PAMPS in a single virus – detection of the genome is crucial to sensing and responding to the presence o

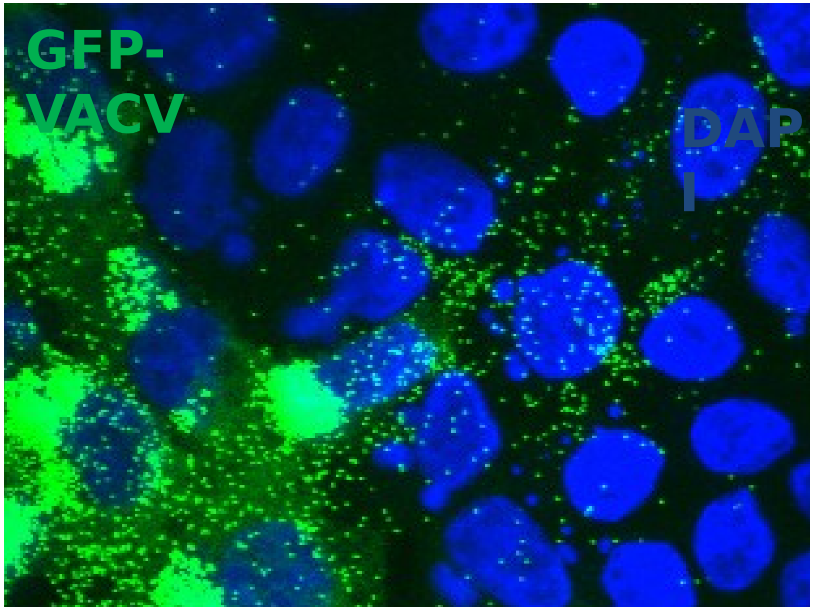


Genome must be exposed for replication to occur

Although this can happen in many different ways depending on the virus

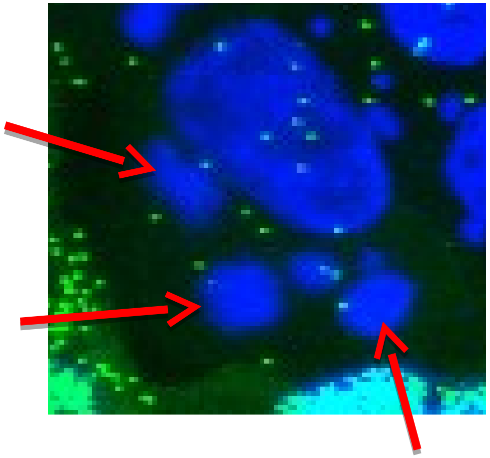
Innate immune response to viral DNA

Viruses – 2 types: DNA (or RNA) genomes – Vaccinia virus is a DNA virus



Vaccinia DNA
'factories'

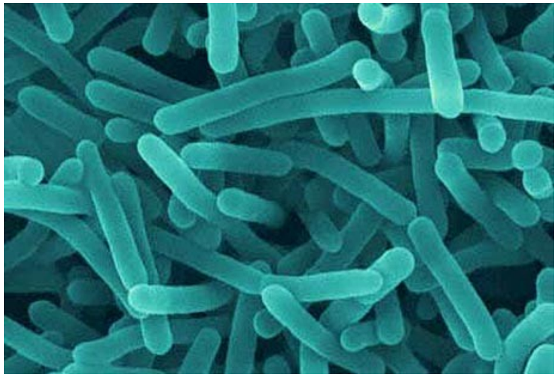
VACV replicates its 200kb DNA genome in the cytoplasm



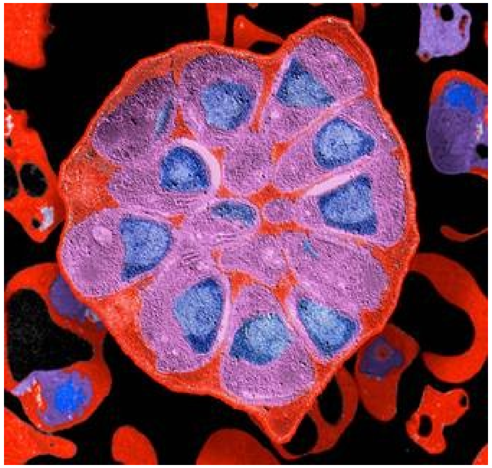
Viral DNA

Intracellular nucleic acid sensing

Not just viruses

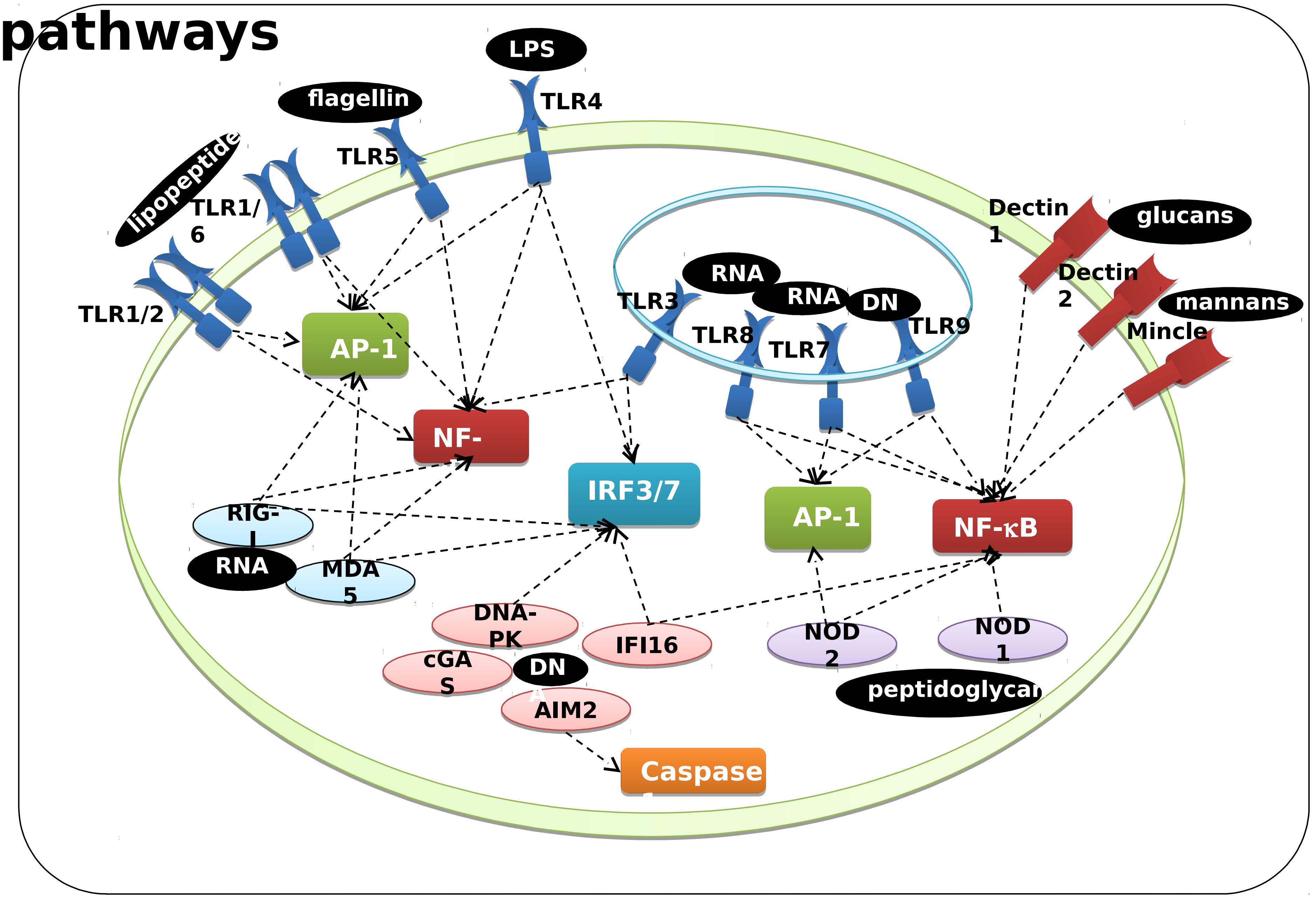


Bacteria – some are intracellular –
Listeria inject its DNA and RNA into cytoplasm following infection

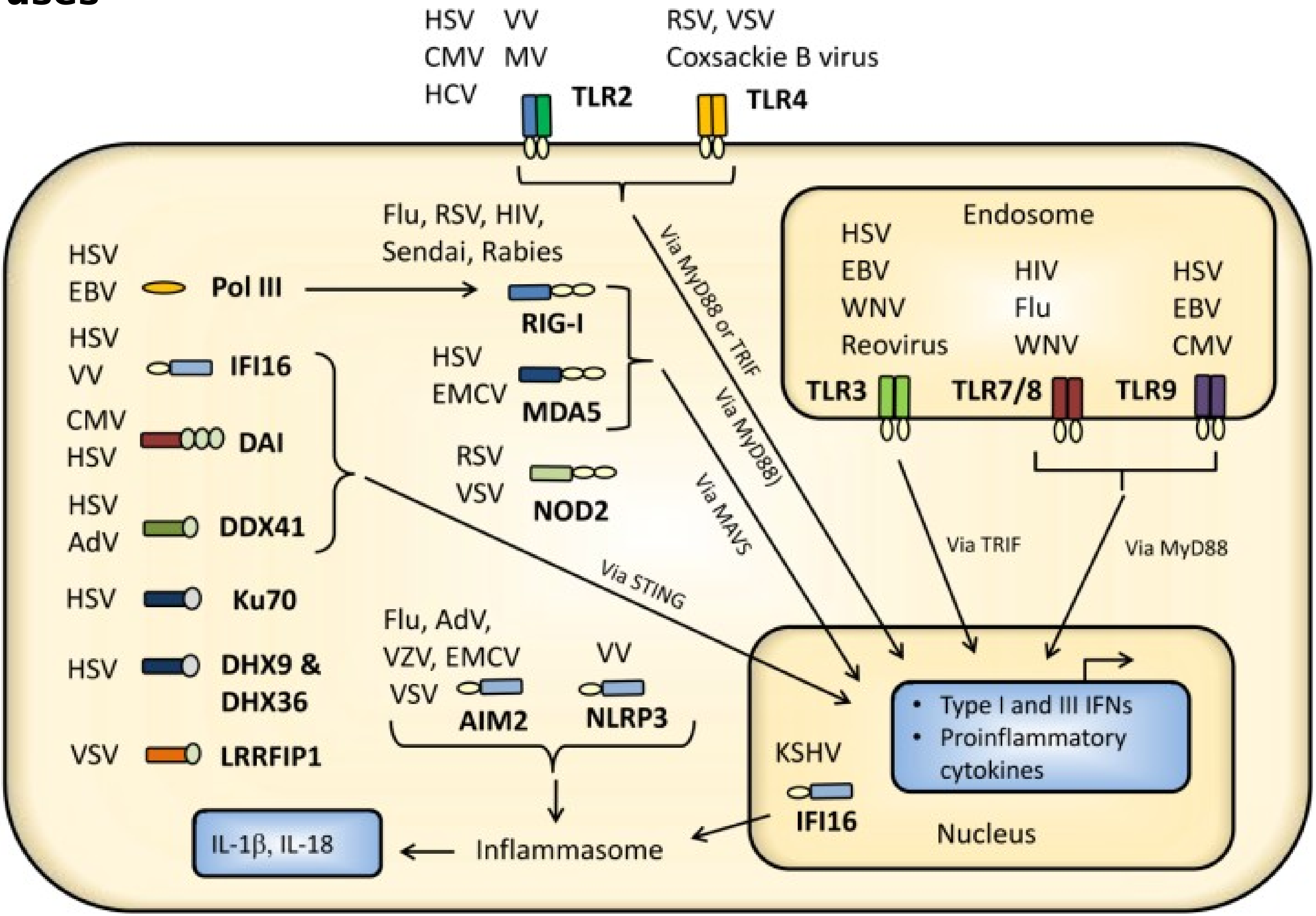


Protazoa – eg plasmodium falciperium
DNA genome sensed by innate immune system

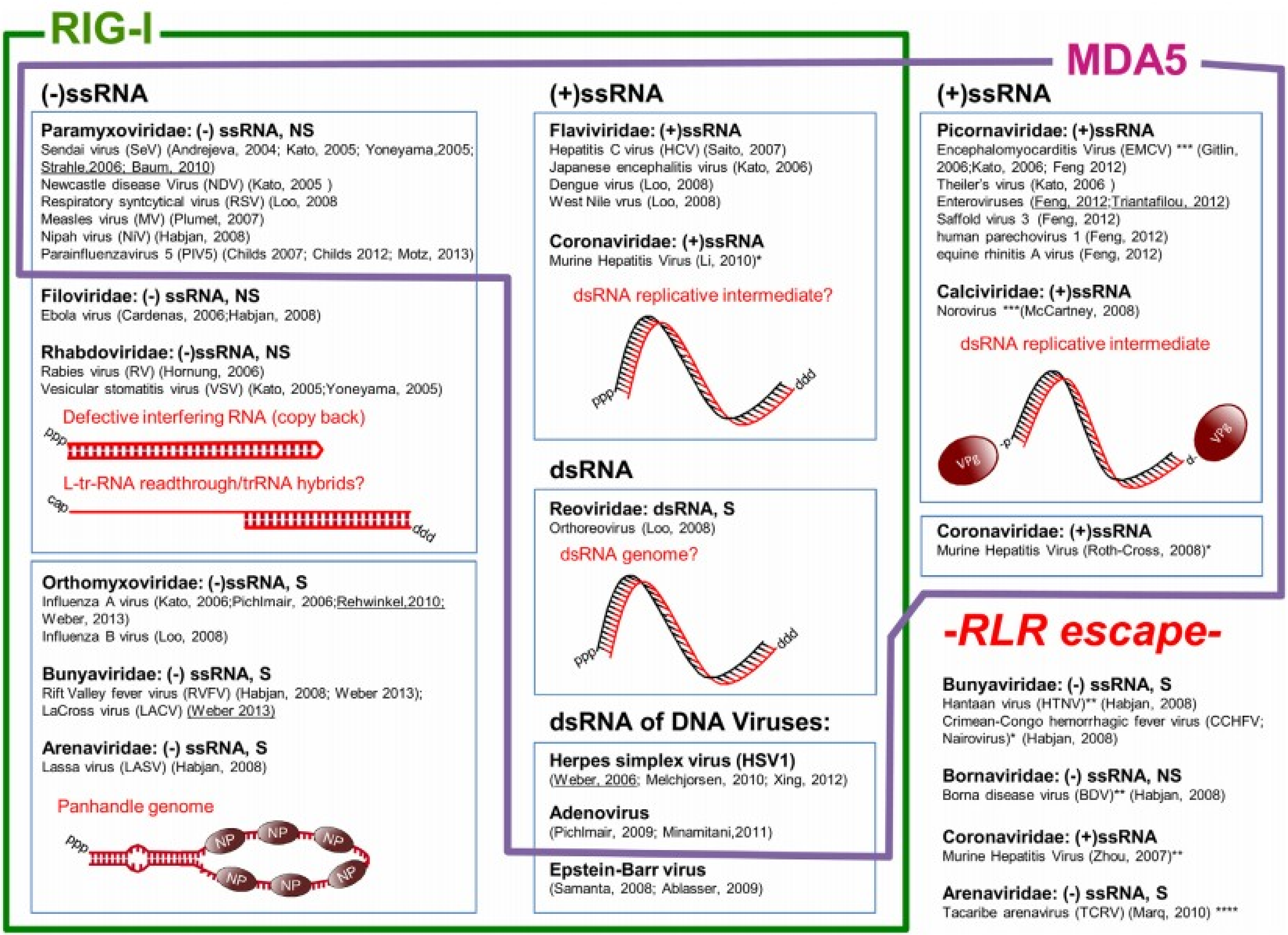
Pattern recognition molecular pathways



PRR sensing of viruses



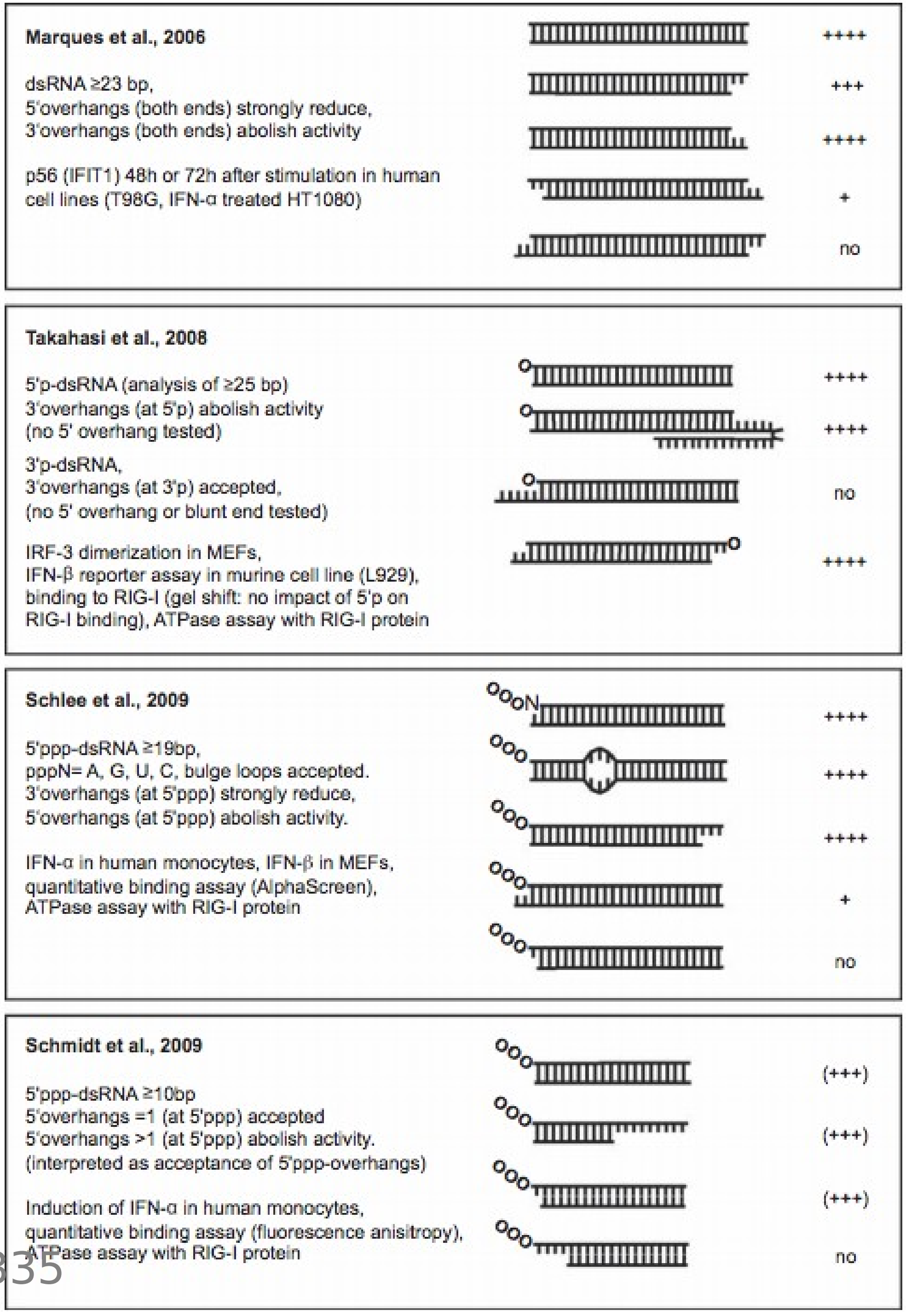
Viral RNA sensing



RIG-I RNA ligands

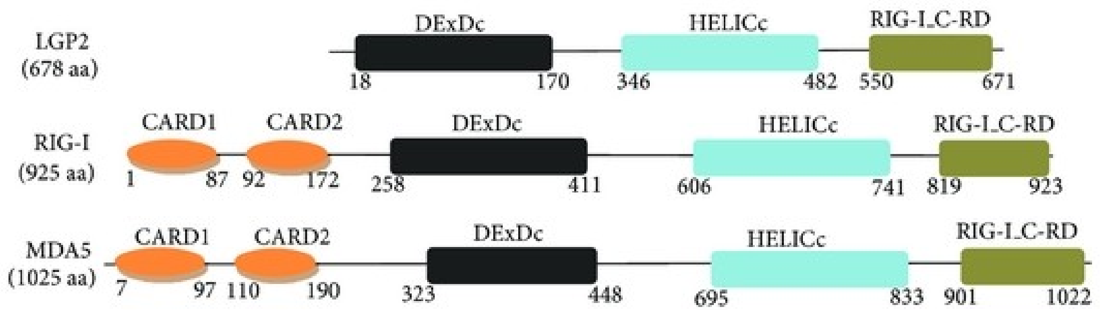
What is the physiological ligand during infection

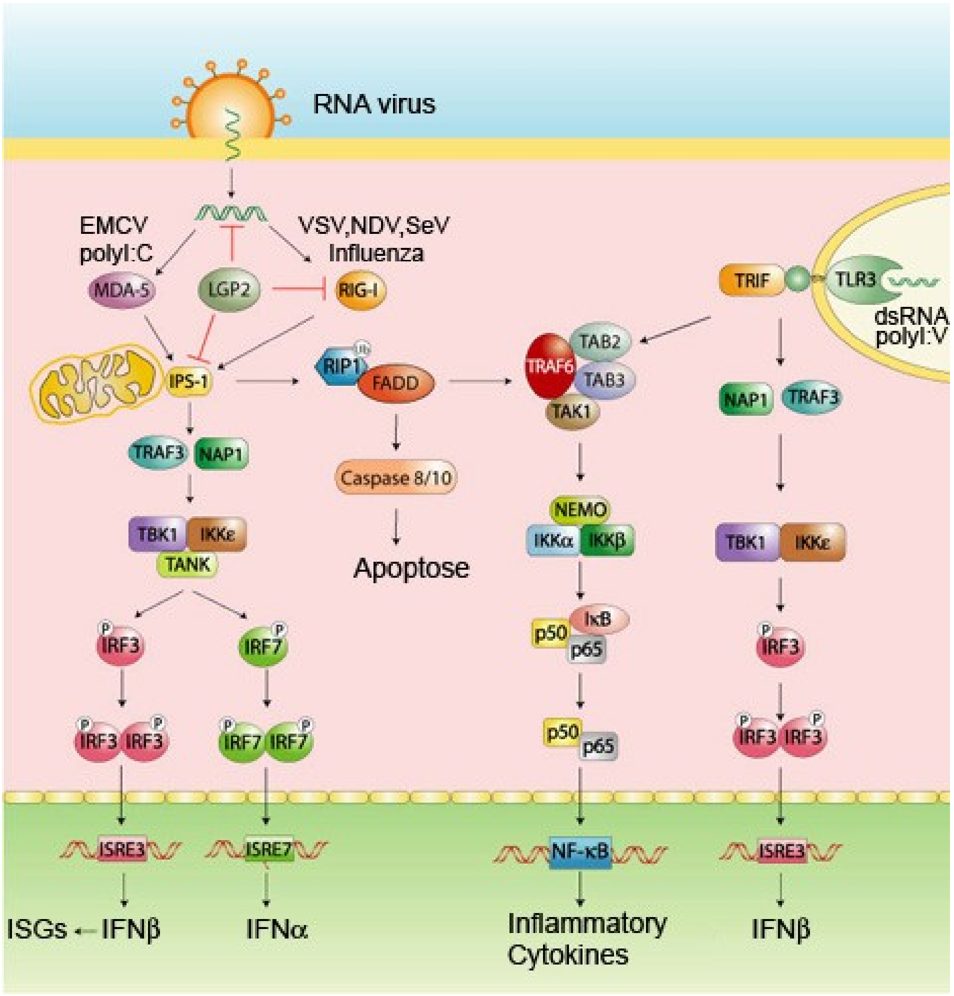
Still an open question



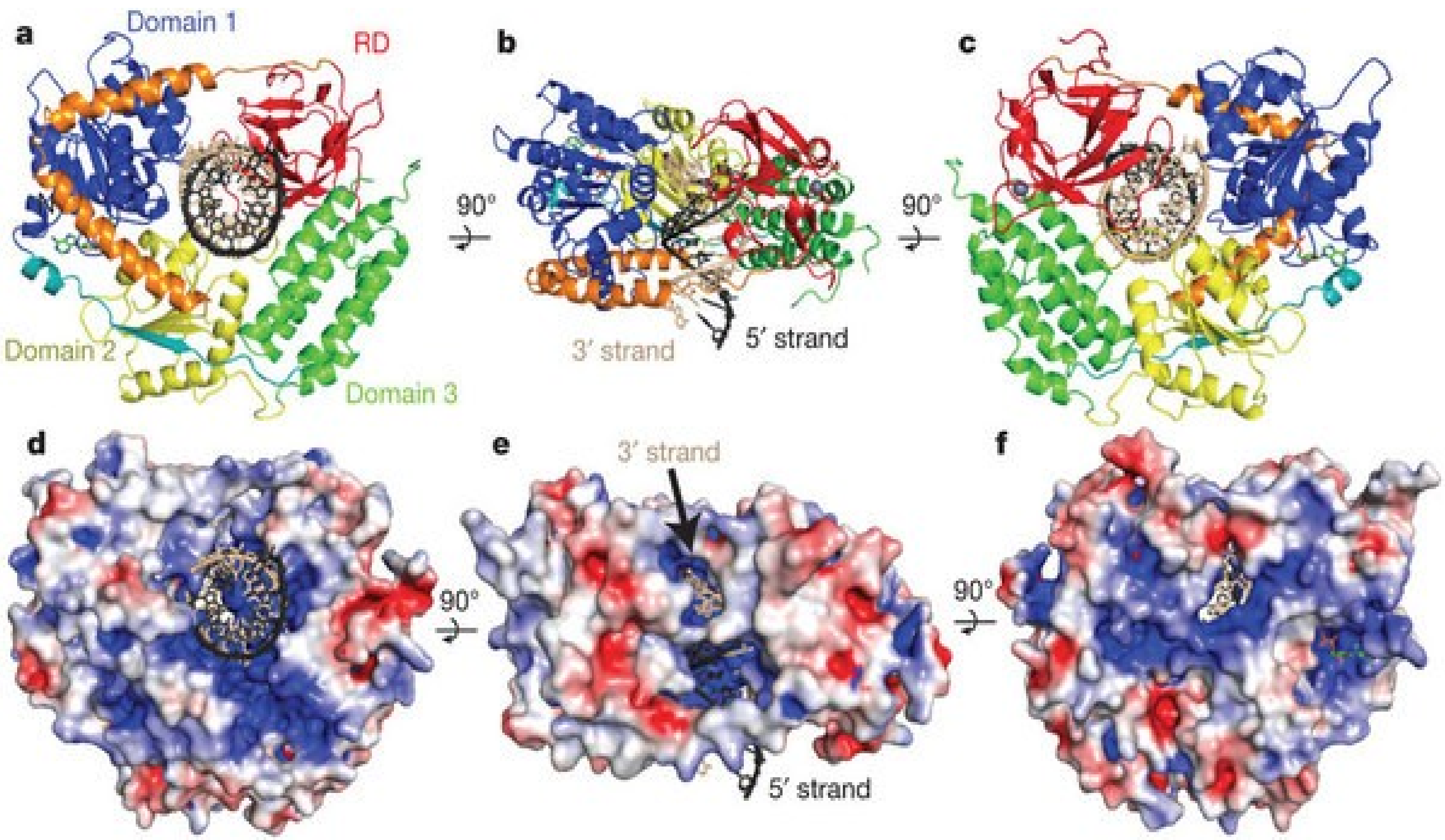
munobiology 218 (2013) 1322–1335

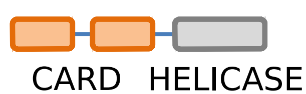
RLR signalling



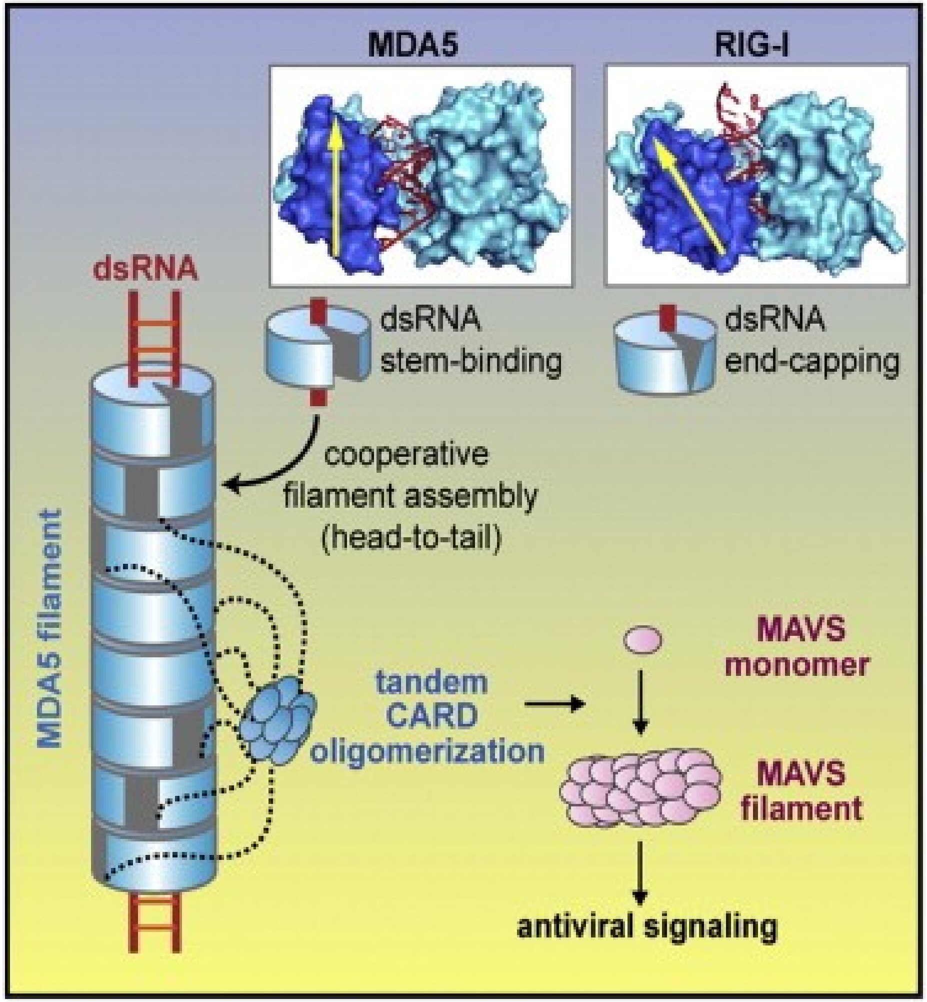


RIG-I recognition of dsRNA



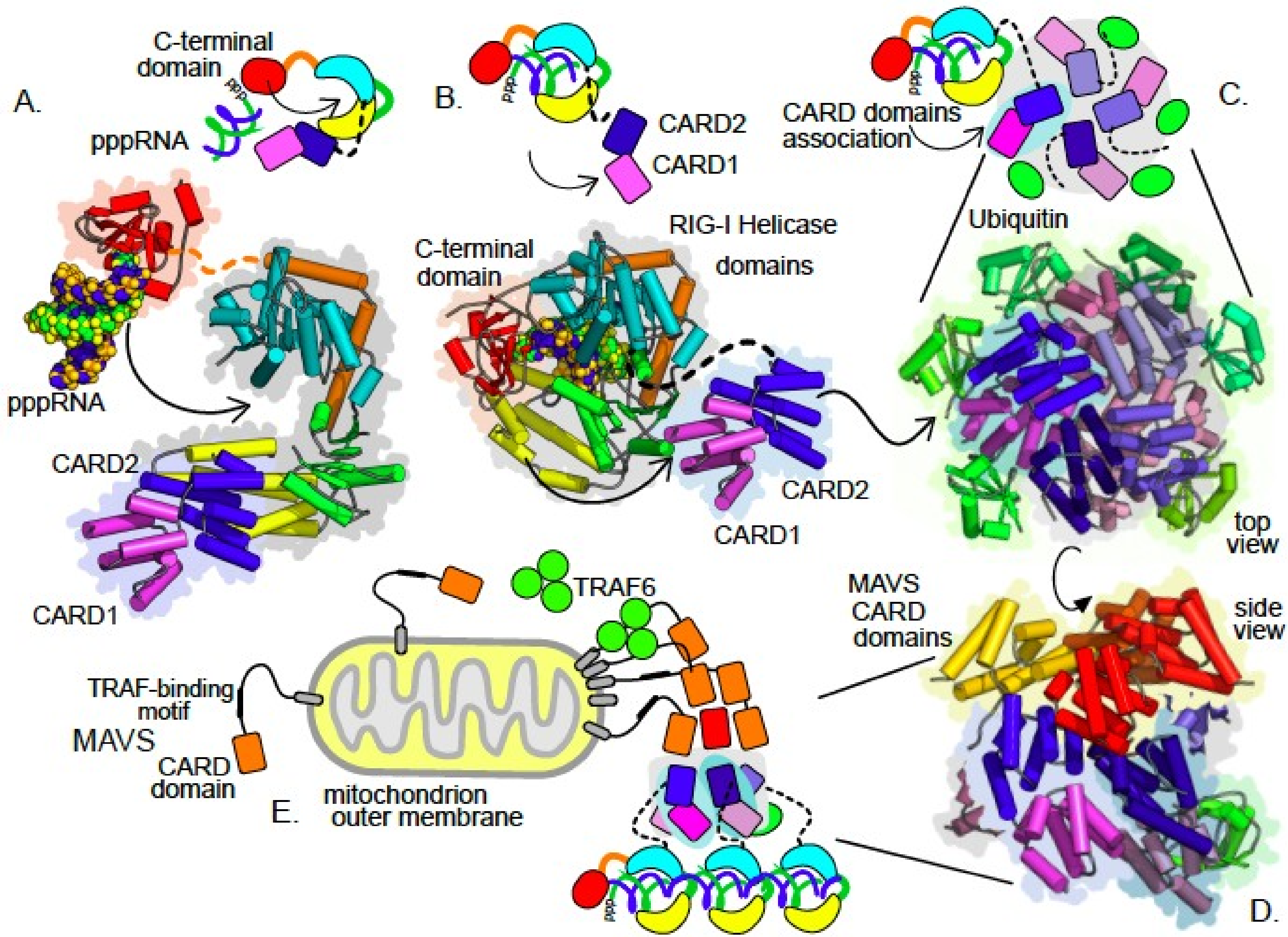


MDA-5 structure

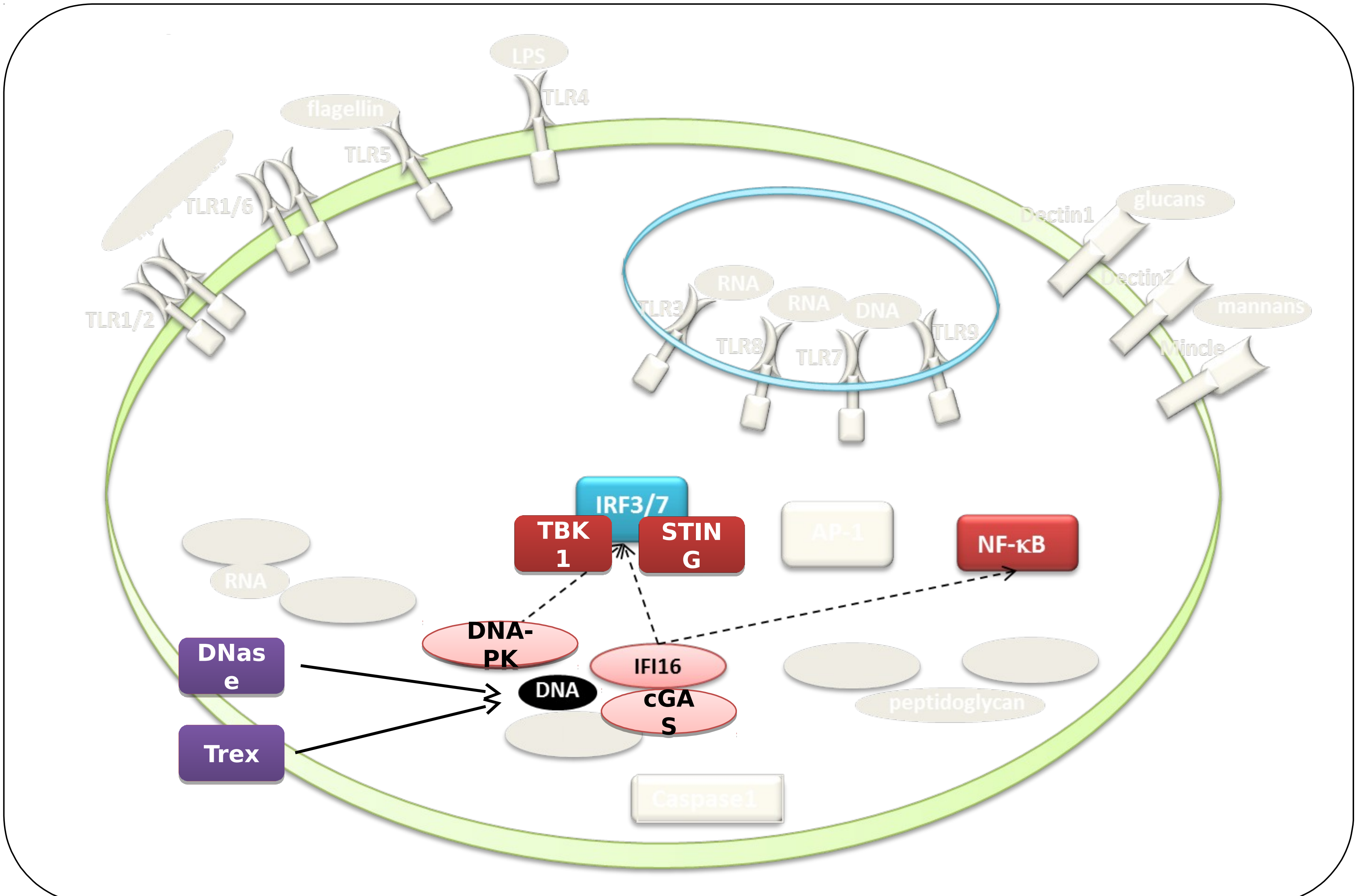


Wu et al Dec 2012 Cell

RLR signalling activation mechanisms

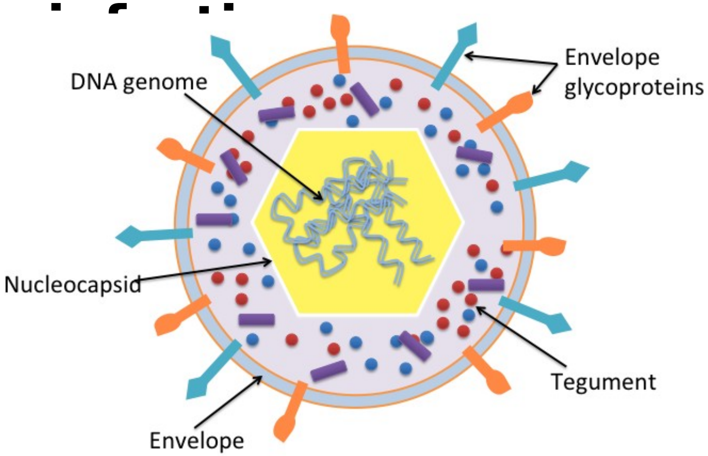


Pattern recognition – DNA

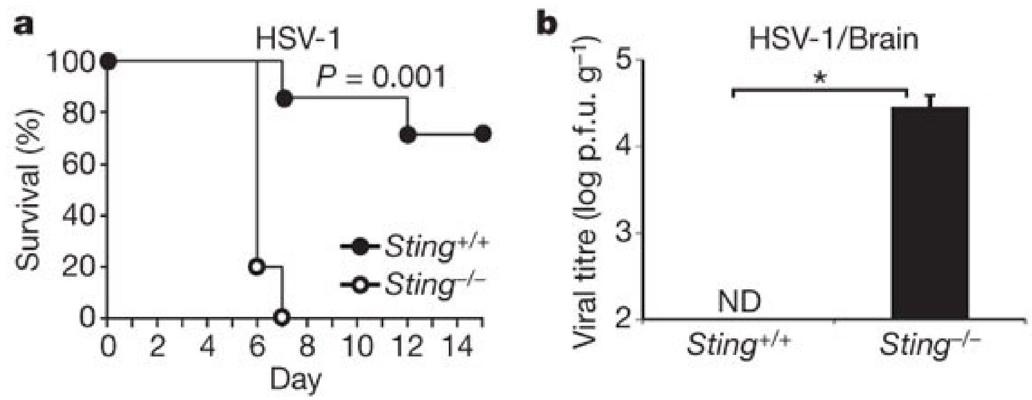


1) Immune response to pathogens

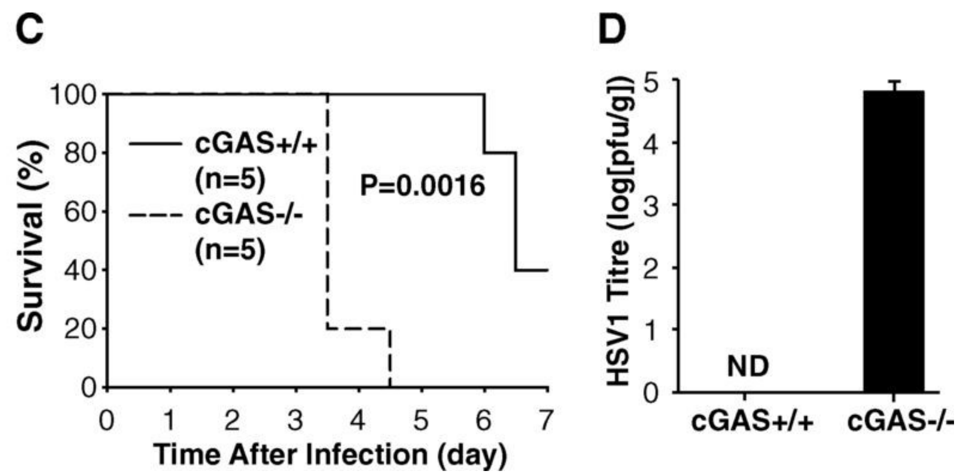
Intracellular innate immune DNA sensing is required for protection against DNA virus



Herpes Simplex virus 1



Nature. 2009 Oct 8;461(7265):788-92.

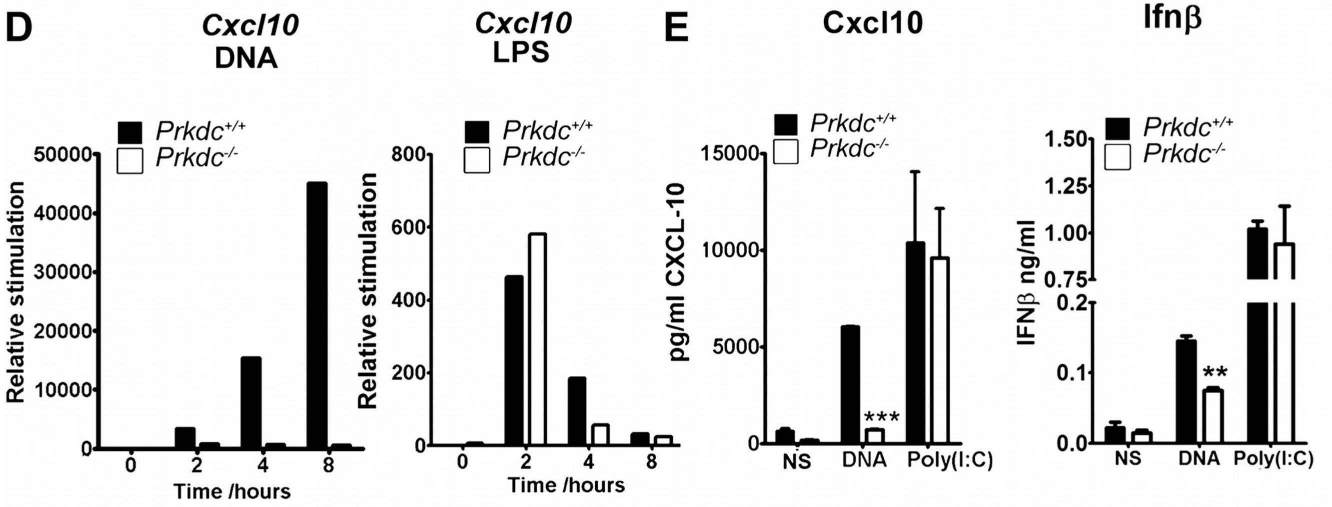


Science 20 September 2013: Vol. 341 no. 6152 pp. 1390-1394

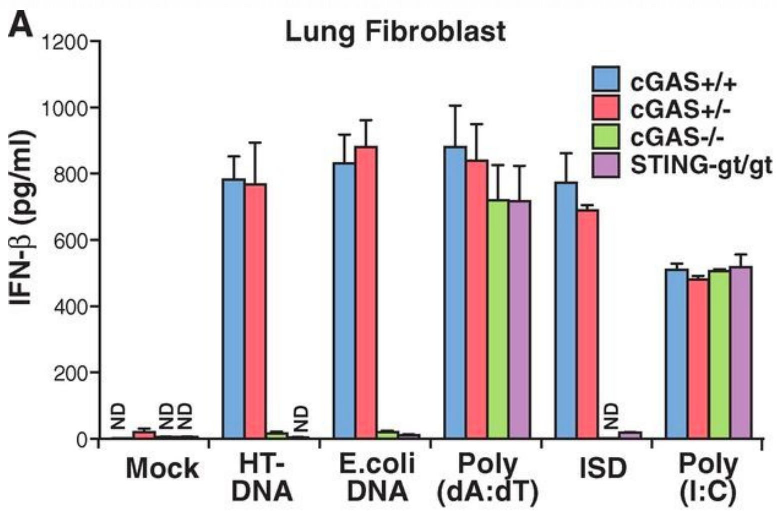
STING -/-, cGAS -/- and IRF3-/- mice are highly susceptible to infection with HSV-1

1) Immune response to pathogens

Intracellular innate immune DNA sensing is required for DNA and DNA-virus induced Interferon and cytokine induction



Elife December 2012; 00047



Cells lacking DNA-PKcs, cGAS, IRF3 or TBK1 do not make cytokines in response to intracellular DNA stimulation or DNA virus infection

Science 2013: Vol. 341 no. 6152 pp. 1390-1394

Enzymatic regulation of DNA levels

Appropriate inflammatory response results in the ability to fight infection <u>without</u> inappropriate responses to self

Hence there is exquisite regulation of these processes

Nucleases regulate levels of circulating and cellular nucleic acids

- DNase I, DNase II and Trex are all responsible for breakdown of DNA

- This effectively stops the innate immune resp
  from starting until a threshold point is reache

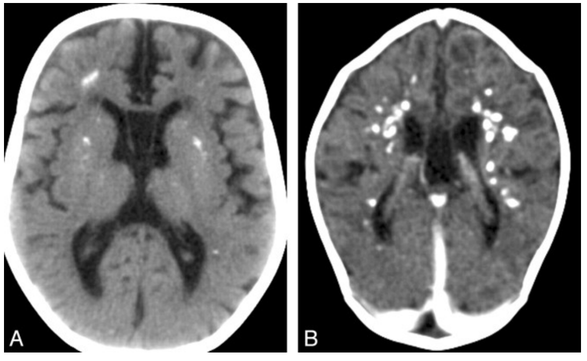
- **Without DNAse, self-DNA triggers inflam**

Dnase II knockout mice get spontaneous TNF driven ‘arthritis’

DNase II$^{-/-}$ Sting$^{-/-}$ do not exhibit signs of arthritis

Autoimmunity from Trex mutation

- **Aicardi–Goutières syndrome –** autoinflammatory disease
- Caused by mutations in Three prime repair exonuclease 1 (Trex)
- Driven by lack of clearance of DNA from apoptotic cells by macrophages

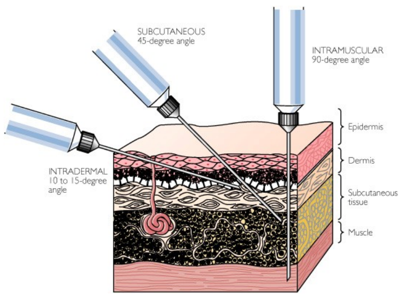


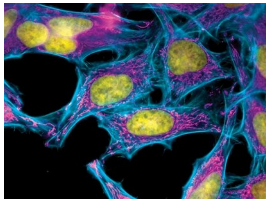
Severe neurological and developmental symptoms in AGS patients

3) DNA vaccination

Plasmid encoding antigen

Electroporation
Gene gun
Intramuscular injection



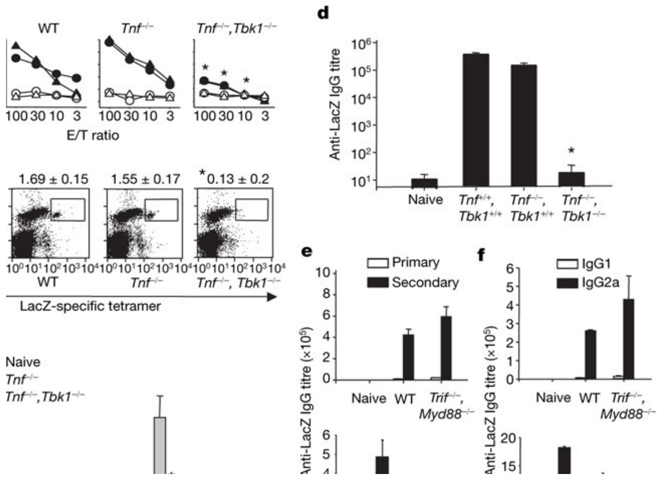


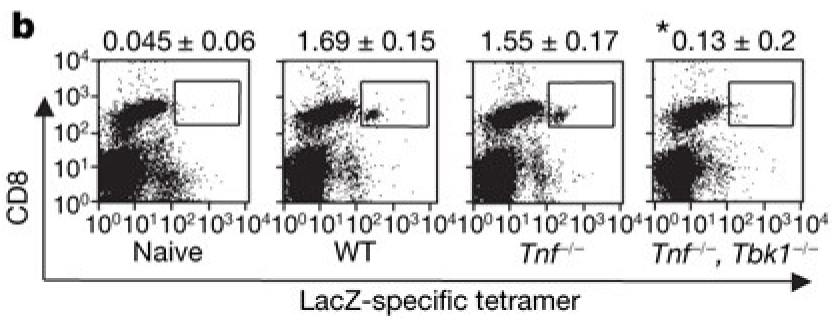
Transcription
Translation
Antigen presentation

Innate immune DNA sensing
Inflammation

Protective immunity to encoded antigen

3) DNA vaccination requires cytoplasmic DNA sensing





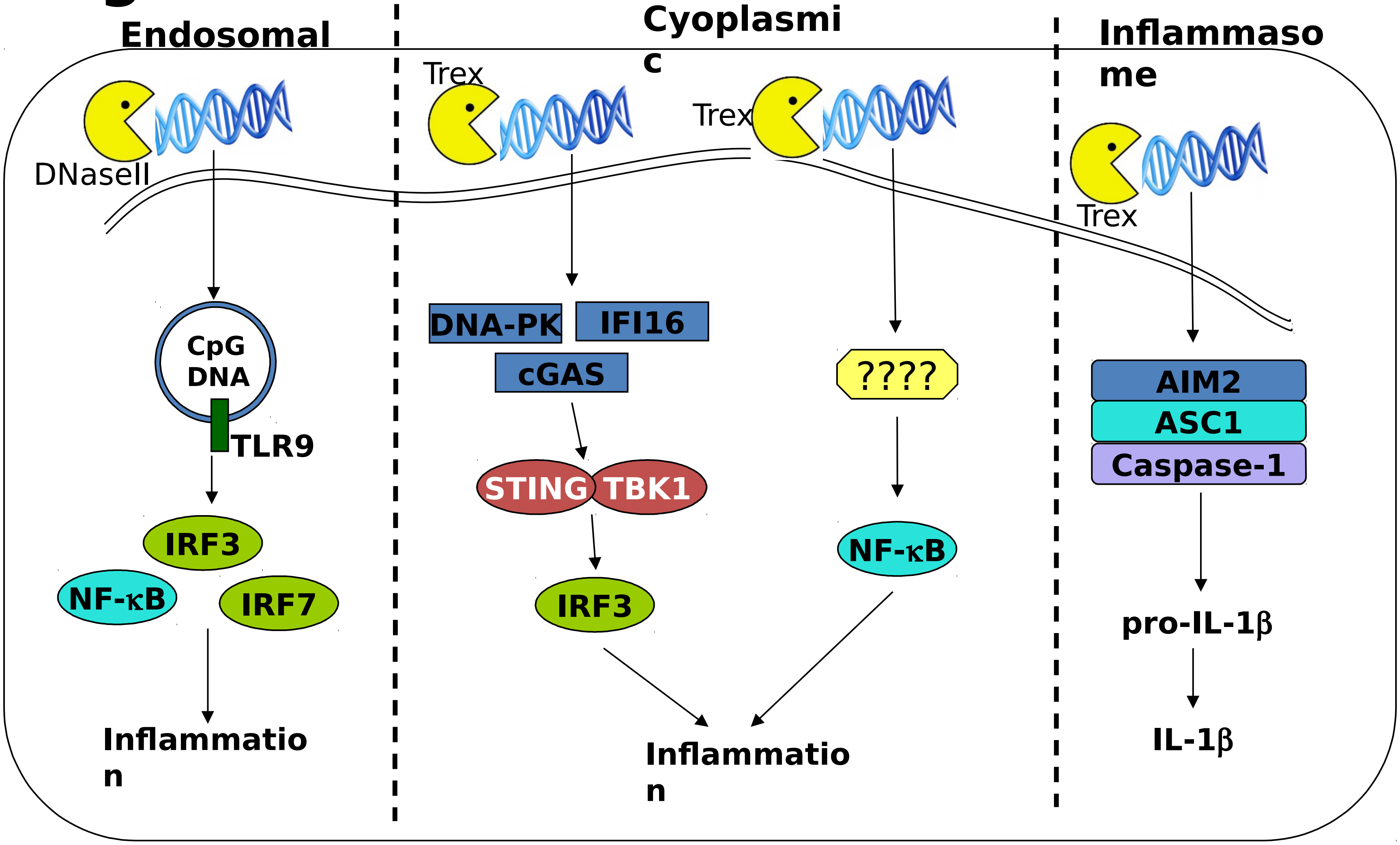
No antigen-specific CTL or IgG response to DNA vaccination in TBK1-/- mouse

Similar data from STING-/- and cGAS-/- mice

For DNA vaccination to result in protective immunity

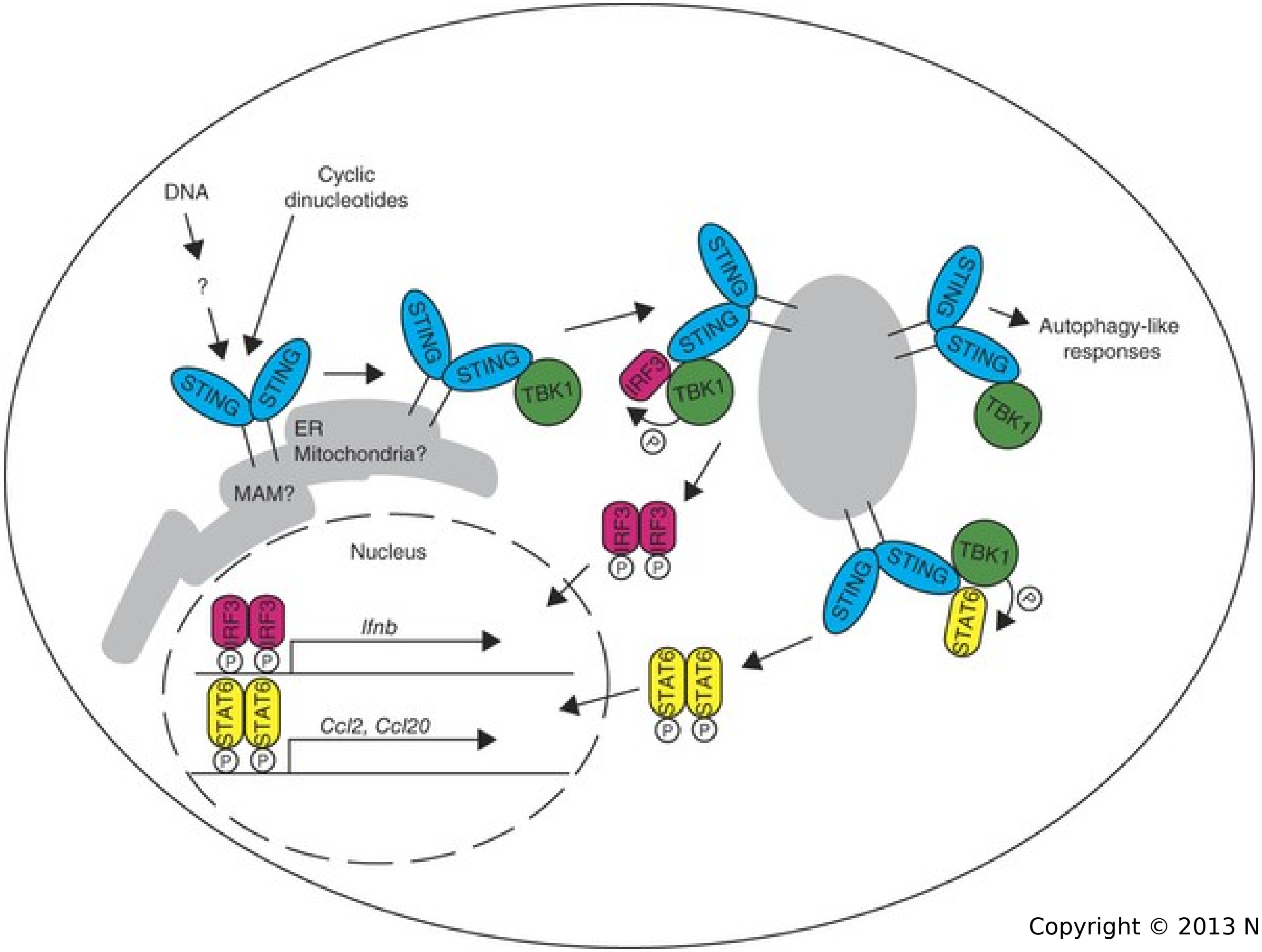
1) Requires entry into cells
2) Requires effective stimulation of intracellular DNA sensing pathways
3) Requires expression of encoded antigen

Molecular mechanisms of pattern recognition – DNA



Molecular mechanisms of DNA recognition

STING: Endoplasmic reticulum (and mitochondrional) resident protein – translocates to the golgi apparatus upon stimulation

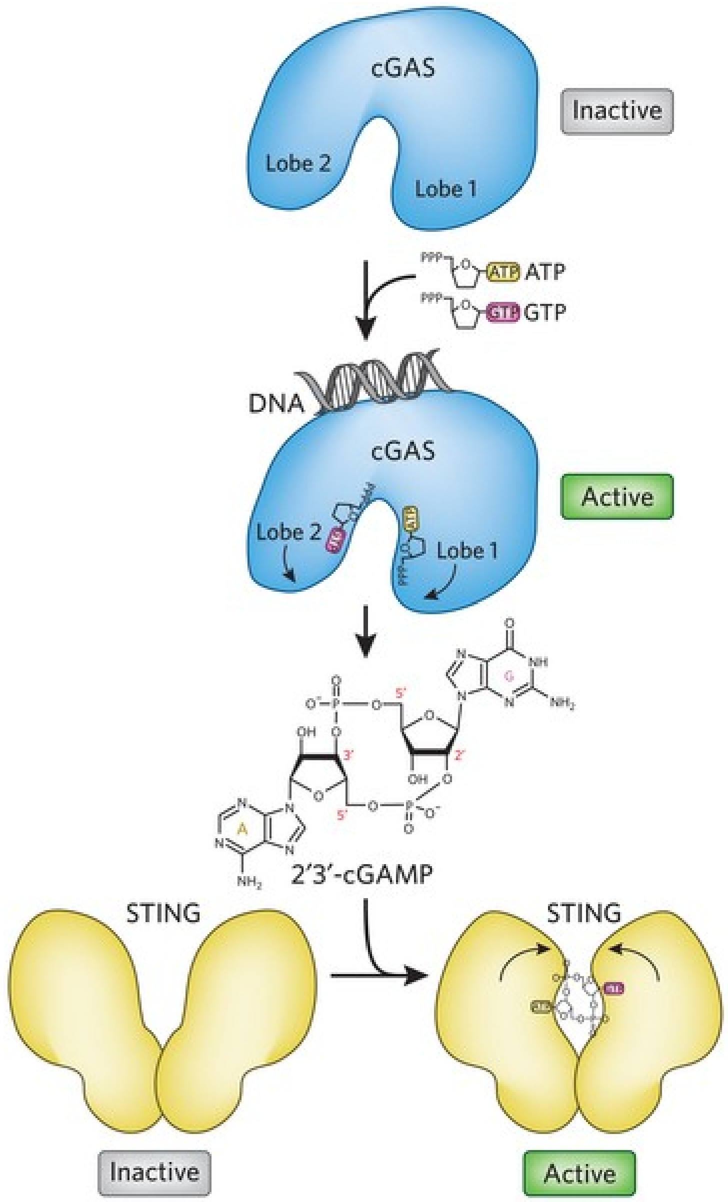


Nature immunology 2013, 14, 19-26

Molecular mechanisms of DNA recognition - cGAS

cGAS syntheses cGAMP

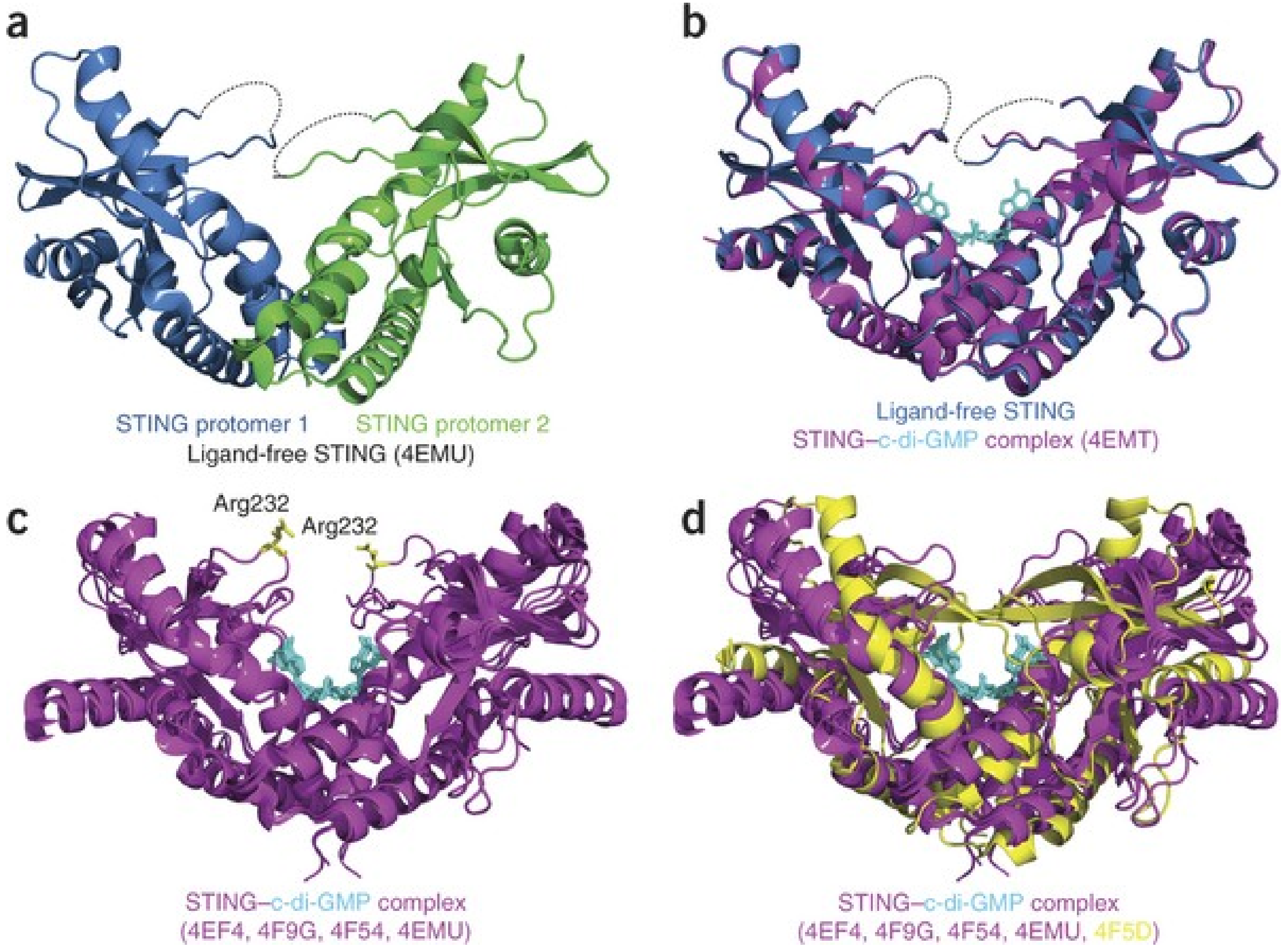
cGAMP activates STING



Nature Chemical biology 2013 9 533-534

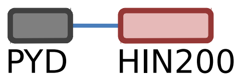
Molecular mechanisms of DNA recognition - STING

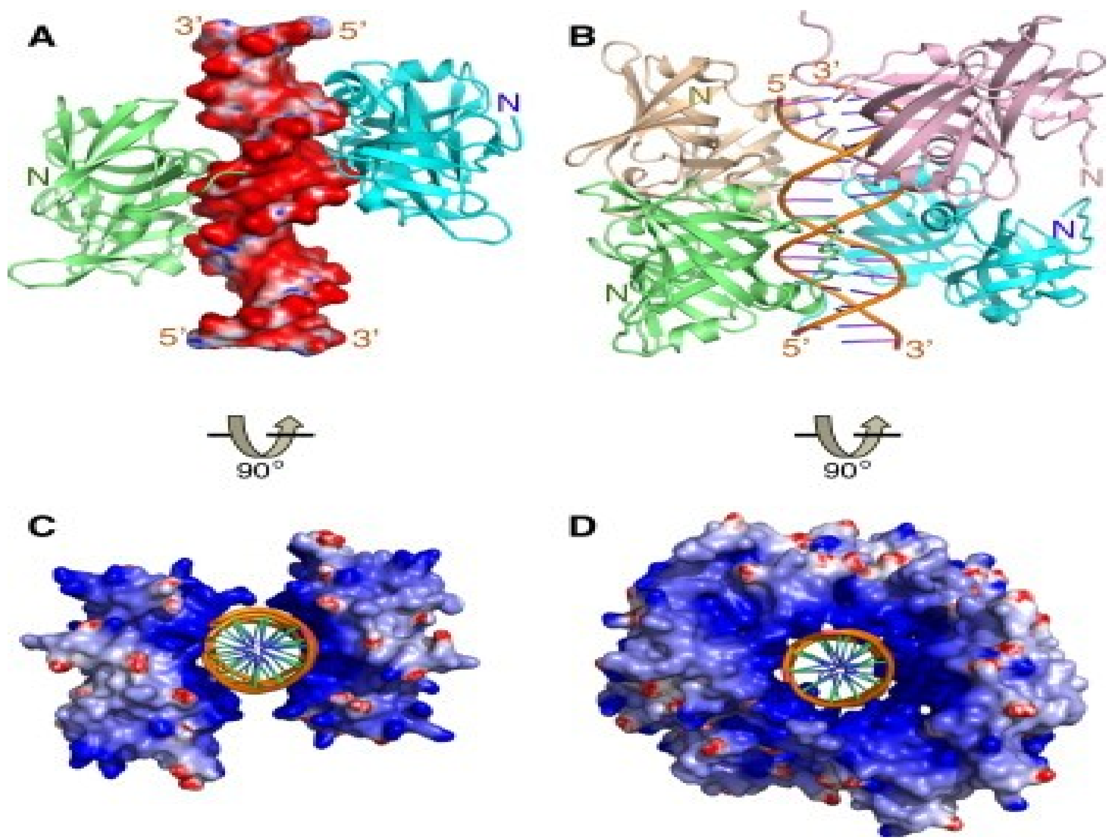
STING structures: binds to cyclic di-nucleotides



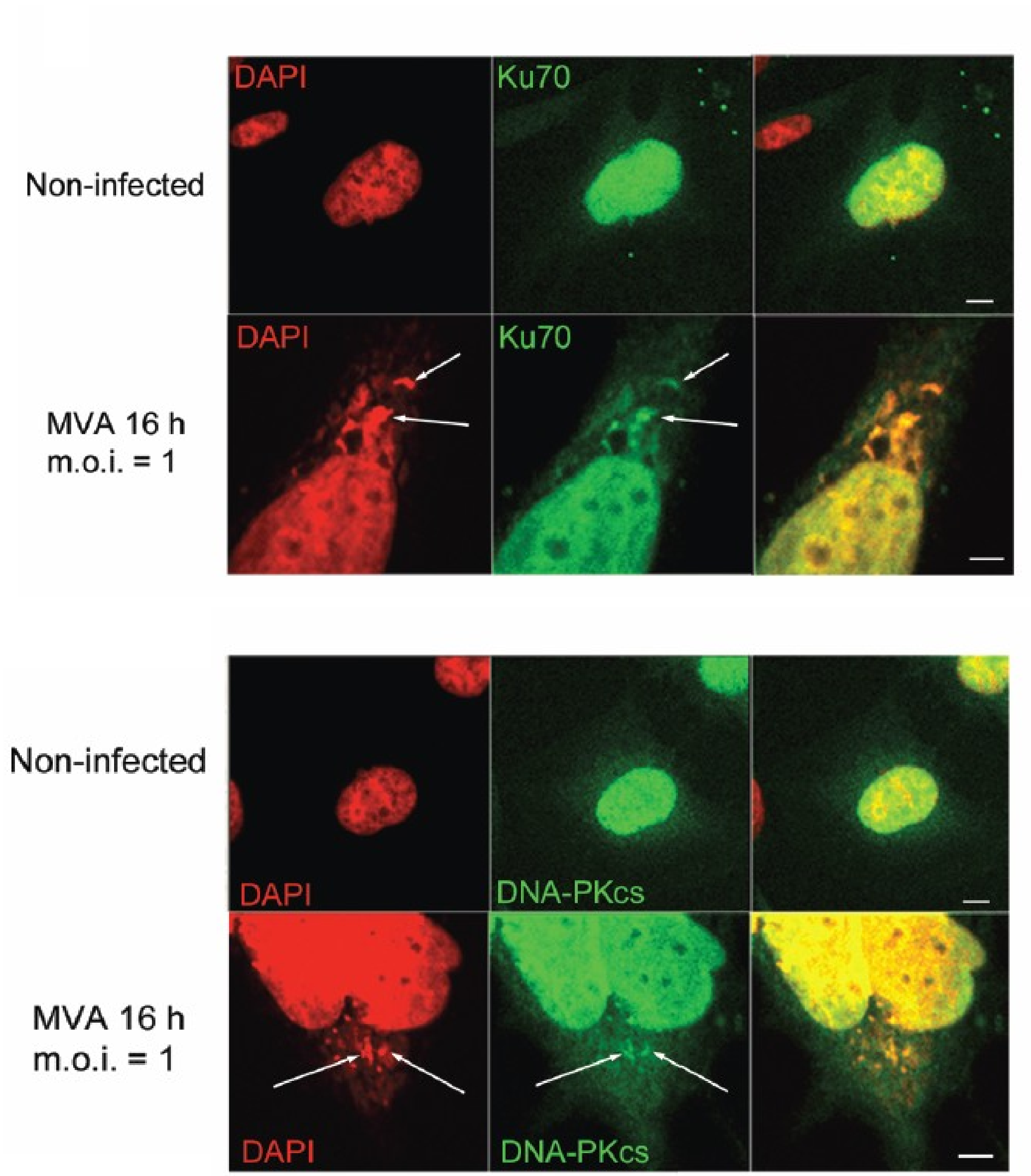
Nature immunology 2013, 14, 19-26

The structure of the AIM2 HIN:DNA complex (crystal form I): AIM2 binds ASC to form an inflammasome and produces IL1 plus cell death



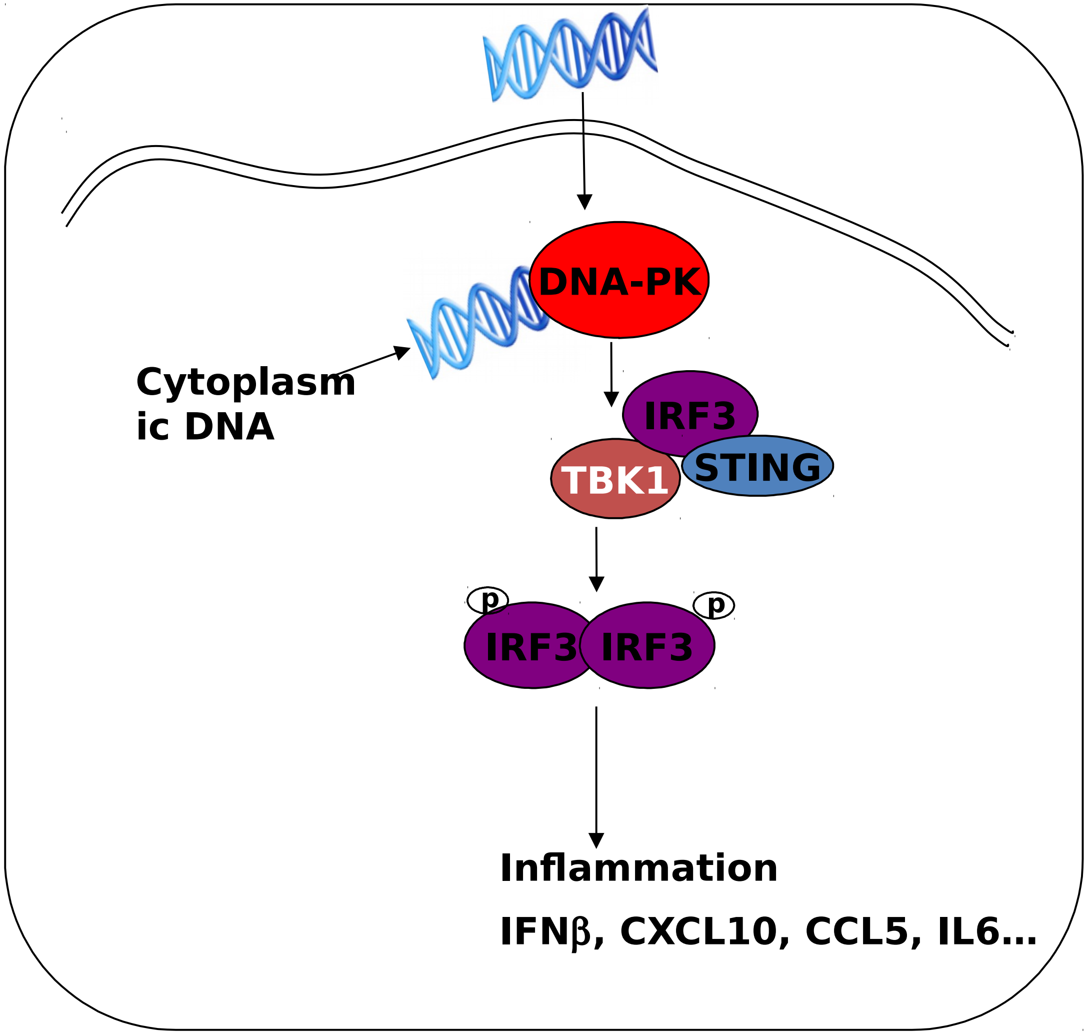


Molecular mechanisms of DNA recognition



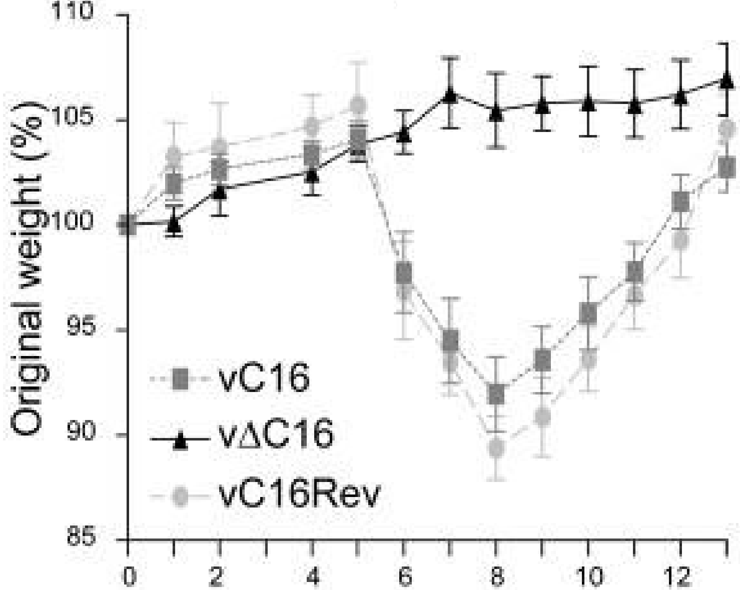
Ku and DNA-PKcs localise to viral DNA factories during MVA infection

DNA-PK / IRF3-dependent signalling



Vaccinia virus protein C16 is a virulence factor

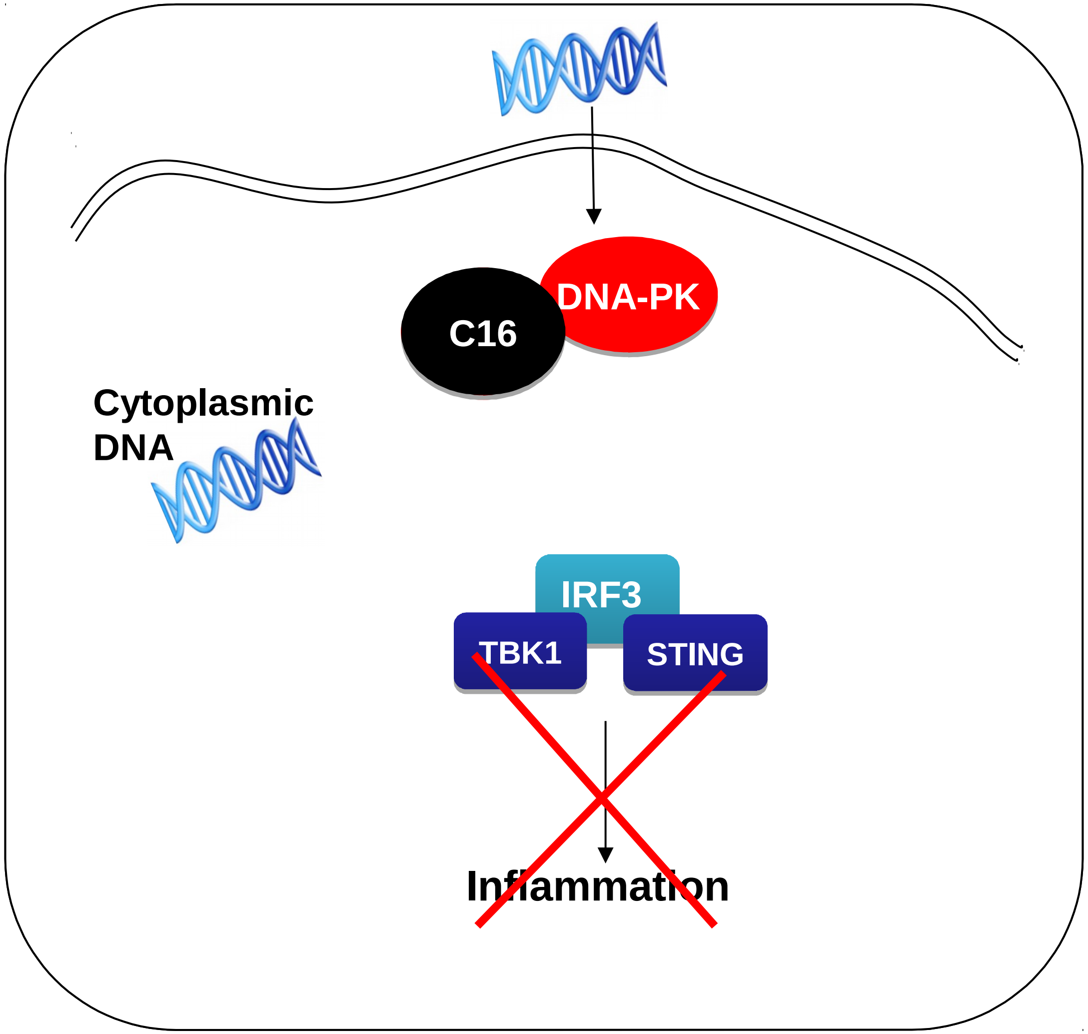
Intranasal infection of mice



Mice infected with VACV ΔC16 lose less weight than those infected with wt virus

Fahy et al (2008)

C16 inhibits DNA-PK dependent DNA sensing



Take Home messages:

Nucleic acids are potently immunostimulatory – when found as non-self structures or found in the inappropriate location

Detection of DNA by innate immune system is crucial for fighting pathogens as well as for warning against tissue damage

Multiple mechanisms of detection of DNA

Cytoplasmic DNA sensing pathways are crucial for fighting infection

Dysregulation of these pathways can lead to autoimmunity, often due to a build up of DNA which triggers inflammatory signalling